THE EFFICACY OF FOUR SPECIES OF SLUG-KILLING NEMATODES ON THE GRAY FIELD SLUG

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Introduction

Slugs are among the most important pests of the grass seed industry in Oregon, and the gray field slug (*Deroceras reticulatum*) is the most damaging species. Current control measures focus heavily on the use of molluscicidal baits, but growers report considerable variation in the efficacy of the most widely used active ingredients, i.e., metaldehyde, iron phosphate, and sodium ferric EDTA (McDonnell and Anderson, 2018). Hence, there is an urgent need to identify and develop alternative control practices for producers in the region.

Biological control offers a compelling option: the use of a pest's natural biological enemies to combat it in the field. Nematode worms in the genus *Phasmarhabditis* are important natural enemies of slugs. In fact, in Europe, a species called *Phasmarhabditis hermaphrodita* is currently used as a commercially available biological control agent labeled under the trade name Nemaslug to successfully manage slug pests in a wide range of crops. It is currently illegal to use Nemaslug in Oregon because the nematode has not been found in the United States. However, over the past 2 years we have surveyed a diverse range of crops for these nematodes and have discovered four species of *Phasmarhabditis* in various locations throughout the Willamette Valley. These discoveries potentially open up Oregon for the use of *Phasmarhabditis* as biological control agents. There is now an urgent need to investigate the infectivity of all four nematodes so the species most lethal to key slug pests, such as the gray field slug (*D. reticulatum*) can be identified for future research and testing as a biological control agent.

Methods

Slug and nematode specimens

Gray field slugs were hand collected in April 2019 from a field of perennial ryegrass grown for seed in Tangent, Linn County, OR, 48 hours before trials were initiated. Specimens were placed into plastic containers (35.9 cm x 20 cm x 12.4 cm), with 30 slugs per container, and were maintained in a growth chamber at a temperature of 18°C and a 12-hour photoperiod. The containers were kept moist with a single paper towel saturated with deionized water. Several slices of organic carrot were placed in each container as food. The paper towels and carrot were replaced three times weekly. Nematodes used in this study were isolated from slugs collected throughout Oregon. DNA identification of these nematodes is ongoing; consequently, they are referred to simply as *Phasmarhabditis* A, B, C, and D for the purposes of this report.

Infectivity trials

The infectivity trial arenas consisted of circular 8-oz plastic containers with 25 g of autoclaved topsoil and perforated lids. The soil was moistened by adding 10 ml of deionized water and mixing thoroughly. We used two nematode treatment rates: 20,000 (low rate) and 40,000 nematodes (high rate) for each species of nematode. Nematodes were placed in 5 ml of deionized water and then pipetted across the soil surface. A nematode-free container was used as a negative control. Six adult gray field slugs were then placed on the soil in the center of the arenas. A total of five replicates were used per nematode treatment and ten replicates for the control. Arenas were maintained in a growth chamber at a temperature of 18°C and a 12-hour photoperiod. A slice of organic carrot was placed in each container for 24 hours on day 8 as a source of slug food. The number of dead slugs was recorded daily for 2 weeks. A slug was deemed to have died when it did not respond to being poked by a blunt needle and/or when its tissue had liquefied. In addition, a dead slug typically would be covered with thousands of nematodes and thus would be visually obvious.

Statistical analysis

It was not possible to normalize the data, so nonparametric statistics were used for analysis. Differences between nematode treatments and controls were investigated using the Kruskal-Wallis test. Posthoc analysis was completed using Dunn's test incorporating the Bonferroni correction for multiple comparisons. Levels of significance corresponding to $P < 0.05$, $P < 0.01$, and $P < 0.001$ were used. All statistical analyses were carried out using IBM SPSS version 24.

Results and Discussion

The analysis showed that there was a significant difference ($P < 0.001$) in median percentage slug mortality between the different nematode treatments and control on days 3 to 14 (Table 1, page 34). No

slug mortality occurred on days 1 and 2. The high rates for both *Phasmarhabditis* A (16% mortality, $P < 0.05$) and *Phasmarhabditis* B (50% mortality, $P < 0.001$) were the first nematode treatments to cause significantly more slug mortality than the controls (0%), with mortality beginning on day 3. The low rates of *Phasmarhabditis* A (100%) and *Phasmarhabditis* B (66%) caused significantly ($P < 0.01$) more mortality versus the control on days 7 and 4, respectively. For *Phasmarhabditis* C, the high (100%, $P < 0.01$) and low (100%, $P < 0.05$) rates resulted in significantly higher *D. reticulatum* mortality compared to the control on day 7 (8%) and 9 (16%) respectively. At no point during this infection trial did *Phasmarhabditis* D cause significantly greater slug mortality compared to the other treatments and control (Table 1).

Complete mortality in all replicates occurred after 5 days, 6 days, 6 days, 8 days, 9 days, and 10 days for *Phasmarhabditis* B high, *Phasmarhabditis* B low, *Phasmarhabditis* A high, *Phasmarhabditis* A low, *Phasmarhabditis* C high, and *Phasmarhabditis* C low, respectively. Complete mortality in all replicates did not occur in the *Phasmarhabditis* D treatments (Table 1).

The high rate of *Phasmarhabditis* B was the only nematode treatment that caused a significantly higher percentage slug mortality when compared to other nematode treatments, and this occurred on days 3, 4, and 5 (Table 1). On day 3, this treatment was significantly ($P < 0.05$) more lethal to *D. reticulatum* than were the *Phasmarhabditis* C low rate, the *Phasmarhabditis* C high rate, and the *Phasmarhabditis* A low rate. It also caused significantly ($P < 0.05$) more slug mortality than the *Phasmarhabditis* C low rate on days 4 and 5 and significantly more mortality than the *Phasmarhabditis* D low rate on day 5.

Conclusion

As pest slugs, including *D. reticulatum,* continue to cause serious damage to crops throughout Oregon, there is a growing need to develop new management tools to help mitigate the losses caused by these pests. This need is particularly pressing given that slug populations are able to proliferate in cropping systems due to several factors, including prohibition of residue burning as a postharvest management option, an increase in no-till and conservation tillage acres, and grower dissatisfaction with the level of control with existing slug baits (McDonnell and Anderson, 2018). Biological control has often been identified as one such tool, and in recent years nematodes in the genus *Phasmarhabditis* have been cited repeatedly as having important potential (Tandingan De Ley et al., 2017). Although past research on *Phasmarhabditis* has focused almost exclusively on *P. hermaphrodita,* primarily due to commercial availability in Europe under the trade name Nemaslug (Rae et al., 2007), other species within the genus have been largely overlooked despite having clear biological control potential. Our data demonstrates that the four species of *Phasmarhabditis* we have discovered in Oregon are also lethal to the key pest, *D. reticulatum,* in laboratory infection trials. However, the extent of mortality and time to cause complete mortality varied greatly among nematode species.

Phasmarhabditis B was the most lethal of the four species investigated here, causing complete slug mortality in all replicates after 5 days with the high inoculation rate and killing significantly more slugs than the controls after just 4 days at both rates. This nematode species should be a prime candidate for additional biological control testing. *Phasmarhabditis* D caused more than 75% slug mortality after 14 days at both rates but was the least virulent of the four species to *D. reticulatum*. In fact, at no point during this infection assay was slug mortality significantly greater with this species of *Phasmarhabditis* compared to the controls, and hence it appears to have the lowest potential for further biological control testing.

References

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Table 1. Mean (+SE) percentage gray field slug mortality recorded daily for the low and high rate treatments of four *Phasmarhabditis* species and controls.[1]

	A High	A Low	B High	B Low	C High	C Low	D High	D Low	Control
Day 3	19.4 ± 3.4[a]	3.2 ± 3.2[b]	43.2 ± 4.2[b,c,d,e]	9.6 ± 3.9	3.2 ± 3.2[d]	3.2 ± 3.2[e]	6.4 ± 3.9	6.4 ± 3.9	0[a,c]
Day 4	36.2 ± 8.1	9.6 ± 3.9	89.8 ± 4.2[f, h]	63.0 ± 11.1[g]	13.0 ± 6.1	6.4 ± 3.9[h]	9.8 ± 6.6	13.2 ± 8.1	1.6 ± 1.6[f,g]
Day 5	73.0 ± 8.5[i]	36.2 ± 9.8	100.0 ± 0[j,k,l]	89.8 ± 4.2[m]	23.0 ± 6.6	6.4 ± 3.9[k]	13.0 ± 6.1	13.2 ± 8.1[l]	1.6 ± 1.6[i,j,m]
Day 6	100.0 ± 0[n]	66.4 ± 15.0	100.0 ± 0[o]	100.0 ± 0[p]	63.0 ± 6.1	29.6 ± 6.4	19.6 ± 6.2	19.6 ± 6.2	6.4 ± 2.6[n,o,p]
Day 7	100.0 ± 0[q]	93.2 ± 4.2[r]	100.0 ± 0[s]	100.0 ± 0[t]	93.2 ± 4.2[u]	59.6 ± 3.9	29.6 ± 3.4	22.8 ± 4.2	9.7 ± 3.6[q,r,s,t,u]
Day 8	100.0 ± 0[v]	100.0 ± 0[w]	100.0 ± 0[x]	100.0 ± 0[y]	93.2 ± 4.2[z]	79.6 ± 6.4	33.0 ± 5.4	26.2 ± 6.8	11.3 ± 3.5[v,w,x,y,z]
Day 9	100.0 ± 0[A]	100.0 ± 0[B]	100.0 ± 0[C]	100.0 ± 0[D]	100.0 ± 0[E]	93.2 ± 4.2[F]	39.8 ± 4.2	36.2 ± 8.1	12.9 ± 3.3[A,B,C,D,E,F]
Day 10	100.0 ± 0[G]	100.0 ± 0[H]	100.0 ± 0[I]	100.0 ± 0[J]	100.0 ± 0[K]	100.0 ± 0[L]	43.0 ± 6.6	46.4 ± 11.1	14.5 ± 2.9[G,H,I,J,K,L]
Day 11	100.0 ± 0[M]	100.0 ± 0[N]	100.0 ± 0[O]	100.0 ± 0[P]	100.0 ± 0[Q]	100.0 ± 0[R]	49.8 ± 13.0	59.8 ± 17.3	17.8 ± 3.0[M,N,O,P,Q,R]
Day 12	100.0 ± 0[S]	100.0 ± 0[T]	100.0 ± 0[U]	100.0 ± 0[V]	100.0 ± 0[W]	100.0 ± 0[X]	59.8 ± 13.6	66.6 ± 14.0	17.8 ± 3.0[S,T,U,V,W,X]
Day 13	100.0 ± 0[Y]	100.0 ± 0[Z]	100.0 ± 0[$]	100.0 ± 0[€]	100.0 ± 0[&]	100.0 ± 0[¥]	76.6 ± 14.6	73.2 ± 11.3	26.2 ± 6.2[Y,Z,$, €,&,¥]
Day 14	100.0 ± 0[£]	100.0 ± 0™	100.0 ± 0[©]	100.0 ± 0[®]	100.0 ± 0[@]	100.0 ± 0[§]	79.8 ± 13.4	83.2 ± 10.6	31.3 ± 8.8[£,™,©,®,@,§]

[1] No slug mortality was recorded on days 1 and 2, and consequently these days were omitted from the table. On specific days, values with the same superscript letter indicate significant differences between the treatments/controls. Pairwise posthoc tests with significance values adjusted by the Bonferroni correction for multiple tests for all time points.

Day 3: H=30.64, N=50, df=8, P=0.000. **a**: Ph High v Control, test statistic=23.70, P<0.05; **b**: Pp High v Ph Low, test statistic=27.90, P<0.05; **c**: Pp High v Control, test statistic=32.30, P<0.001; **d**: Pp High v Pc High, test statistic=27.90, P<0.05; **e**: Pp High v Pc Low, test statistic=27.90, P<0.05

Day 4: H=34.19, N=50, df=8, P=0.000. **f**: Pp High v Control, test statistic=34.10, P<0.001; **g**: Pp Low v Control, test statistic=29.40, P<0.01; **h**: Pp High v Pc Low, test statistic=29.30, P<0.05

Day 5: H=39.94, N=50, df=8, P=0.000; **i**: Ph High v Control, test statistic=28.30, P<0.01; **j**: Pp High v Control, test statistic=35.70, P<0.001; **k**: Pp High v Pc Low, test statistic=31.80, P<0.05; **l**: Pp High v Pu Low, test statistic=28.80, P<0.05; **m**: Pp Low v Control, test statistic=32.10, P<0.01

Day 6: H=42.75, N=50, df=8, P=0.000; **n:** Ph High v Control, test statistic=34.20, P<0.001; **o**: Pp High v Control, test statistic=34.20, P<0.001; **p**: Pp Low v Control, test statistic=34.20, P<0.001

Day 7: H=45.41, N=50, df=8, P=0.000; **q:** Ph High v Control, test statistic=33.00, P<0.01; **r**: Ph Low v Control, test statistic=28.00, P<0.01; **s**: Pp High v Control, test statistic=33.00, P<0.01; **t**: Pp Low v Control, test statistic=33.00, P<0.01; **u**: Pc High v Control, test statistic=28.00, P<0.01

Day 8: H=45.41, N=50, df=8, P=0.000; **v:** Ph High v Control, test statistic=31.40, P<0.01; **w**: Ph Low v Control, test statistic=31.40, P<0.01; **x**: Pp High v Control, test statistic=31.40, P<0.01; **y**: Pp Low v Control, test statistic=31.40, P<0.01; **z**: Pc High v Control, test statistic=25.80, P<0.05

Day 9: H=46.15, N=50, df=8, P=0.000; **A:** Ph High v Control, test statistic=30.30, P<0.01; **B**: Ph Low v Control, test statistic=30.30, P<0.01; **C**: Pp High v Control, test statistic=30.30, P<0.01; **D:** Pp Low v Control, test statistic=30.30, P<0.01; **E**: Pc High v Control, test statistic=30.30, P<0.01; **F**: Pc Low v Control, test statistic=24.30, P<0.05

Day 10: H=47.60, N=50, df=8, P=0.000; **G:** Ph High v Control, test statistic=29.35, P<0.01; **H**: Ph Low v Control, test statistic=29.35, P<0.01; **I**: Pp High v Control, test statistic=29.35, P<0.01; **J:** Pp Low v Control, test statistic=29.35, P<0.01; **K**: Pc High v Control, test statistic=29.35, P<0.01; **L**: Pc Low v Control, test statistic=29.35, P<0.01

Day 11: H=41.50, N=50, df=8, P=0.000; **M:** Ph High v Control, test statistic=27.55, P<0.01; **N**: Ph Low v Control, test statistic=27.55, P<0.01; **O**: Pp High v Control, test statistic=27.55, P<0.01; **P:** Pp Low v Control, test statistic=27.55, P<0.01; **Q**: Pc High v Control, test statistic=27.55, P<0.01; **R**: Pc Low v Control, test statistic=27.55, P<0.01

Day 12: H=42.68, N=50, df=8, P=0.000; **S:** Ph High v Control, test statistic=28.20, P<0.01; **T**: Ph Low v Control, test statistic=28.20, P<0.01; **U**: Pp High v Control, test statistic=28.20, P<0.01; **V:** Pp Low v Control, test statistic=28.20, P<0.01; **W**: Pc High v Control, test statistic=28.20, P<0.01; **X**: Pc Low v Control, test statistic=28.20, P<0.01

Day 13: H=39.53, N=50, df=8, P=0.000; **Y:** Ph High v Control, test statistic=26.70, P<0.01; **Z**: Ph Low v Control, test statistic=26.70, P<0.01; **$**: Pp High v Control, test statistic=26.70, P<0.01; **€:** Pp Low v Control, test statistic=26.70, P<0.01; **&**: Pc High v Control, test statistic=26.70, P<0.01; ¥: Pc Low v Control, test statistic=26.70, P<0.01

Day 14: H=34.64, N=50, df=8, P=0.000; £**:** Ph High v Control, test statistic=24.00, P<0.01; **™**: Ph Low v Control, test statistic=24.00, P<0.01; ©: Pp High v Control, test statistic=24.00, P<0.01; ®**:** Pp Low v Control, test statistic=24.00, P<0.01; **@**: Pc High v Control, test statistic=24.00, P<0.01; §: Pc Low v Control, test statistic=24.00, P<0.01